STAINING FOR PERCENTAGE AM COLONIZATION

(INVAM, Giovannetti and Mosse, 1980)

Materials

250 or 500 um sieve
10% KOH
2% HCl
bunsen burner
beakers
tissue cassettes, vials or test tubes
0.05% Direct Blue stain [33 ml lactic acid, 33 ml glycerol, 33 ml water, 0.5 g Direct Blue 15 (Sigma D2535)]

Methods

1) Wash roots with tap water over sieve to remove soil.
2) Remove a subsample to determine dry weight.
3) Remove another subsample and place in tissue cassettes, vials or test tubes for staining.
4) Roots may be stored in water at 4^{o}C like this for a week or so before staining. (If they need to be stored for a longer period, place the roots in a 1:1:1 lactic acid: glycerol: water solution at 4^{o}C.)
5) Bring 10% KOH solution to a boil and pour over roots. Incubate for 5 min.
6) Remove KOH and rinse with tap water 5 times.
7) Add RT 2% HCl and incubate for 5 min.
8) Meanwhile, bring 0.05% Direct Blue stain to boil.
9) Remove HCl solution and add stain. Incubate 5 min.
10) Remove stain and store in water at 4^{o}C. (Note: Stain is a hazardous waste, dispose of properly.)
11) If a long term storage is desired, store in 1:1:1 acid:glycerol:water solution at 4^{o}C in air tight containers. Monitor for fungal contamination and if roots become to destained, repeat steps 7-10.
12) Determine percentage colonization via grid-line intersect method outlined in Giovannetti and Mosse, 1980.